

assistants. It should have a board of directors representative of prominent and professional people in all sections of the state. The organization of groups in many communities should thus be insured, around which would revolve the educational activities in fields related to mental hygiene and social welfare. Through the payment of dues it should be possible to maintain financial support. A mental hygiene division could be developed into one of the strongest sections of the organization.

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FEMALE SEX HORMONES AND MENSTRUATION*

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RECENT developments in the recognition of various hormones concerned with the physiology of the female sex organs have served to open a fertile field for investigation. Although a great deal yet remains obscure, marked progress has been made and it would seem that one may anticipate for the near future an important advance in our understanding of the functional disturbances of menstruation. The following brief review presents some of the more outstanding observations that have been recorded, and an attempt is made to demonstrate the interrelationship between the hormones of the ovaries and the anterior hypophysis, the application of animal studies to the human, and the importance of recent discoveries in the treatment of menstrual disorders.

THE MENSTRUAL CYCLE

Menstruation has always been a subject of great interest to mankind, but it is only within a comparatively few years that there has been any realization of the significance of this process and the anatomic changes which accompany it. For this we are indebted to the epoch-making histologic investigations of Hirschmann and Adler, R. Schroeder, R. Meyer, and others, who demonstrated the existence of a definite recurring cycle of events in the ovary and in the endometrium. All work on "sex hormones" must be construed in keeping with these anatomical transformations and seek to explain the mechanism by which they are brought about.

The normal menstrual cycle takes approximately twenty-eight days to run its course and it is customary for clinical purposes to regard the

day of onset of menstruation as the first day of the cycle. On the fourth or fifth day, that is immediately after the termination of the menses, are found definite processes in ovaries and endometrium. In one of the ovaries a primordial follicle is developing and maturing into a graafian follicle, and the endometrium, which was left with but a thin basal layer, is proliferating and increasing in depth with the formation of a superficial functional layer. At about the fourteenth day the graafian follicle has reached its maximum stage of development so that it ruptures and the ovum escapes to be taken up by a ciliary current and carried down the fallopian tube to the uterus. The ruptured follicle then undergoes a complete transformation to become a *corpus luteum*, and this is accompanied with a set of characteristic changes in the histology of the endometrium. Before ovulation occurs the glands of the functional layer are straight with a clearly outlined lumen, the individual cells of a low columnar type, and the stroma dense and made up of spindle-shaped connective tissue cells. With the development of the corpus luteum, however, the endometrial glands assume an altogether different appearance; they become markedly swollen and tortuous, the lumen irregular, and they are filled with a secretion of mucus. The stroma has also changed in that it is edematous, laden with glycogen, coursed with numerous dilated blood vessels, and the individual cells have hypertrophied and assumed a polygonal shape so that they have become identical with the "decidual cells" of pregnancy.

One of two things may now occur. If conception has taken place, the fertilized ovum embeds itself in the endometrium which has been prepared for its reception, and the fully developed yellow body in the ovary continues its existence as the "corpus luteum of pregnancy." If, however, pregnancy does not occur, a series of degenerative changes set in. The corpus luteum begins to regress, and the tissues of the endometrium undergo necrosis. As this progresses the surface mucosa is cast off, blood vessels are torn across, and the hemorrhage of menstruation ensues. The desquamation takes about three days for its completion, and finally the whole cavity of the uterus remains covered with but the thin basal layer. A new primordial follicle is now launched forth on its career, the endometrium again begins to proliferate, and the same series of changes is repeated during the succeeding four weeks.

THE HORMONE FACTORS OF MENSTRUATION

It has long been recognized clinically that there is a vital intercommunication between the various glands of internal secretion. This is also true in regard to the ductless glands which are responsible for menstruation, and this fact must be constantly kept in mind. Although such distant structures as the thyroid and the adrenal must play some part, little is understood as to their exact rôle and in this short review their influence must be overlooked. The attention of recent investigators has been directed mainly to the effects

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of substances which are purported to arise from (a) the ovary, and (b) the anterior lobe of the hypophysis.

A. *Ovary*.—Although it was assumed that the ovary had an internal secretion, it is only since the work of Frank, Allen, Doisy, Corner, Hisaw, Laqueur, Loewe, Zondek, Aschheim, Clauberg, and others, that any progress has been made in its identification. It now seems fairly well proven that at least two hormones are manufactured by the ovary, and each plays a distinct rôle in the events of the menstrual cycle.

1. *Estrin*.—The first of these hormones to be recognized has received a great deal of publicity in various ways, and for some time was considered as the only internal secretion of the ovary. It has been given a vast array of names which have served to complicate the subject, for it seems that every investigator and every manufacturing concern considers it a duty to burden the already existing terminology with a new contribution. It is known, for instance, as the ovarian hormone, the female sex hormone, the follicular hormone, the estrous hormone, estrin, folliculin, menformon, theelin, tokokinin, thykinin, estrogen, amniotin, and so forth.

The basic characteristic of this hormone is that it is an "estrous-producing" and a "growth-producing" substance, and when injected into castrated rats, mice, or guinea-pigs it induces histologic changes which are normally found during the period of estrus and a growth of the uterus and mammary glands. In the human it is produced both by the graafian follicle and corpus luteum, in fact, its production seems to increase gradually during the course of the menstrual cycle and to reach its maximum just before the onset of menstruation. Its effect in the human is to induce a growth of the uterus, turgor, and a proliferation of the endometrium. It is thus the first substance to induce an endometrial reaction immediately after menstruation and is the factor concerned with the "interval" type of mucosa, but it continues to function and to an increasing extent up to the time of menstruation.

Estrin is apparently widely distributed in nature, and it would seem that it must be considered as a basic biologic substance rather than merely a hormone specific for the ovary. It has no species specificity and has been demonstrated in the follicle fluid, corpus luteum and placenta of many different birds and animals, and has likewise been found to act upon many species. It has been demonstrated in the blood, urine, and bile of both men and women, and in numerous types of plants from the yeast organism up (buds of female willows, yellow pond lily, potatoes, sugar beets, rice, wheat, etc.). As Frank states, it would seem that we are dealing with a substance which in lower forms of plant life is merely a growth-stimulating principle, and "that gradually as we trace it through the higher plants it becomes more and more localized in the specifically generative portions of the plant. Finally in the animal kingdom the hormone is increasingly centralized in the ovary and its action is more and more

specifically limited to the generative tract." Doisy has recently succeeded in obtaining it in a crystalline form (Theelin).

2. *Progestin*.—The action of estrin, however, is insufficient to account for all the endometrial changes that precede menstruation, and the work of Corner, Hisaw, and their associates has demonstrated the existence of a hormone of the corpus luteum (termed "progestin" by W. Allen, Corner, et al.) which is entirely distinct from estrin in its effects. There is as yet but little experimental data as to the influence of this substance in the higher mammals, but this can be readily deduced from certain animal investigations and the histologic studies previously outlined. It has been shown that an extract of this second corpus luteum factor can induce uterine reactions characteristic of early pregnancy, such as progestational proliferation of the rabbit's endometrium, or the special sensitization of the guinea-pig's uterus necessary for the production of experimental deciduomata; it can produce "pseudo-pregnancy" in the vagina of rats and mice, and cause a relaxation of the guinea-pig's symphysis pubis, a reaction which normally occurs in this animal during pregnancy; it can inhibit ovulation, and finally its administration in sufficient doses can maintain pregnancy until full term in rabbits spayed at the eighteenth hour after mating. A very important observation in regard to these experiments is the fact that it is absolutely necessary to give preliminary doses of estrin before the required effects can be induced with progestin. These results cannot be obtained with either estrin or progestin alone, nor by giving progestin first and estrin second. It is a "one-two" reaction, as Hisaw points out, which cannot be reversed.

The same procedure is found in the reactions of the endometrium during the normal menstrual cycle, the estrin stimulating proliferation during the first half of the cycle. During the second half there is the combined effect of both estrin and progestin, the influence of the latter resulting in those specific changes (secretory phase of glands; decidual cells) which make up the "premenstrual" or "pregnoid" type of endometrium and which is essential for the reception and development of the fertilized ovum.

B. *Anterior Hypophysis*.—The existence of a functional interrelationship between the anterior lobe of the hypophysis and the ovaries has been demonstrated by many clinical and anatomic studies. For instance, as a result of hypophysectomy or destruction of the gland by disease there results an atrophy of the pelvic organs; during pregnancy there is a characteristic enlargement of the anterior lobe, and this also occurs following castration although there is a histologic difference between the two conditions. It remained, however, for Evans and Long in 1921 to produce definite changes in the ovaries of experimental animals by the administration of certain extracts of the anterior lobe. By this means they produced an inhibition of the onset of estrus in immature animals and a marked enlargement of the ovaries due to the formation of very abundant lutein

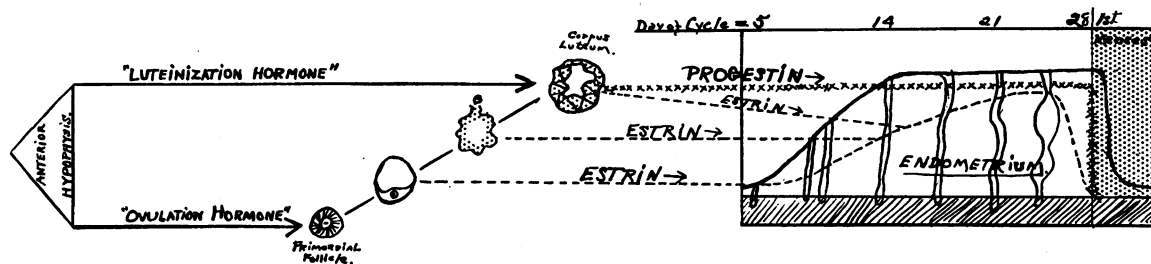


Diagram illustrating the relation of the "sex hormones" of the anterior hypophysis and the ovaries to the endometrial changes of menstruation. The broken line (---) shows the effect of estrin and its production in increasing amounts till just before menstruation, while the line (xxxxx) demonstrates the influence of progesterin during the second half of the cycle. See text. (The representation of the endometrium is after R. Schroeder.)

tissue about the eggs in unruptured normal follicles and in atretic follicles. Later work (Smith and Engle; Aschheim and Zondek) with direct implantations of fresh anterior pituitary gland tissue did not corroborate these findings but produced exactly opposite results, namely, the induction of precocious sexual maturity in immature animals and the development of many graafian follicles with the accompanying "estrin" effects on the uterus and vagina. Both these studies have received abundant confirmation from numerous sources, and successful transplants have been obtained not only from numerous species, including humans, but from males and females of all ages from intra-uterine life and adolescence to adults and senile individuals.

It thus seems that we have here a number of seemingly contradictory findings regarding the action of the anterior lobe on the ovaries. The answer, however, lies in the method of preparing extracts, and it is probable that there are two hormones arising from this gland. In the first place, there is the "ovulation" or "maturity" or "follicle ripening" hormone which stimulates the ovary of an immature animal in such a way as to produce the ripening of numerous follicles and hence the elaboration of estrous effects on the pelvic organs. The second, or "luteinization" factor, acts on the ovaries in such a way as to stimulate a production of lutein tissue, with the elaboration of the second ovarian (or corpus luteum) hormone and hence an inhibition of estrus or ovulation with premenstrual or pre-gravid changes in the uterus.

These effects have been considered as specific for the anterior lobe, but they have also been obtained with other tissues from pregnant individuals, for instance, from the urine, placenta, and amniotic fluid.

SUMMARY

On the basis of these investigations a theory has been evolved which seeks to explain the means by which the changes of normal menstruation are brought about. There is, in the first place, the influence of the anterior pituitary "follicle ripening hormone" on the development of a graafian follicle in the ovary and the consequent elaboration of estrin which in turn stimulates the uterus to growth, turgor, and the proliferation of the basal layers of the endometrium. The second anterior pituitary factor coming into play then causes a "luteinization" of the cells of the ruptured follicle and the formation of a

second ovarian hormone (progesterin) which acts on the endometrium to produce the changes characteristic of the premenstrual phase, or in the case of gestation, to decidual transformation. (See accompanying diagram.)

There are doubtless numerous deficiencies in such a theory, for it fails to explain many details regarding ovulation and it necessarily will have to be altered as new facts are brought to light. It also apparently gives no explanation for the occurrence of menstruation itself, but the answer is to be found in the fact that menstruation does not occur from direct stimulation but is due to degenerative changes which set in because of the removal of hormonal stimulation. This is shown not only by the histological demonstration of a regression of the corpus luteum as menstruation is initiated, but also by actual experiments in monkeys. Allen and others have been able to produce a marked proliferation of the endometrium in the macacus rhesus by prolonged administration of estrin, and have found that within a few days after discontinuing the injections there is a prolonged and profuse loss of blood with a desquamation of the functional layers of the endometrium.

LABORATORY TESTS

On the basis of the experimental studies outlined above it has been possible to develop simple laboratory tests to determine the presence of estrin or anterior pituitary hormone in the blood, urine or tissues of patients. These procedures represent a very distinct advance in the study of endocrine disorders and there are many possibilities for this method of investigation.

In the case of estrin the test consists in the injection of the substance to be examined into an adult spayed mouse, and it is considered positive when the vaginal smear shows the presence of cornified cells and an absence of leukocytes within sixty hours (Allen-Doisy test). Frank and Goldberger have developed a method to examine blood with this test and the studies which have been carried out in this manner are proving not only of interest from the standpoint of physiology, but also in the consideration of certain menstrual disorders.

The test for the anterior pituitary hormone (or hormones) is performed by the injection of the substance to be tested into immature mice or rats and noting the ovarian changes which are set up in about one hundred hours. The finding of graafian follicles denotes the presence of the

"follicle-ripening hormone," and structures with lutein cells the presence of the "luteinization hormone" (Aschheim-Zondek test). The main application of this procedure has been as a "pregnancy test," the demonstration of large amounts of the "luteinization hormone" in the urine of women being considered as positive. This test is a most accurate one and has been found correct in from 97 to 98 per cent of cases.

The Aschheim-Zondek test may also be used in the study of certain endocrine conditions. Extensive studies on the presence of unduly large amounts of one or other of these anterior lobe factors in the blood of patients have been carried out in the Stanford Gynecological Laboratory, and many interesting results have been obtained which it is thought will prove of clinical assistance.

THERAPY

A. *Ovary*.—In considering the question of the therapeutic use of sex hormones one is met with such a mass of contradictory reports that it is difficult to determine whether the development of biologically active substances has really been a marked advance. The problem may be considered from various standpoints: (a) the preparations available; (b) the dosage; (c) the time of administration; (d) the indications; and (e) the results.

(a) *Preparations*.—The past few years have seen a sincere attempt on the part of manufacturing concerns to cooperate with laboratories and to offer to the profession preparations which have been tested by recognized biologic standards. There are now many preparations of estrin available, and usually the potency is given as so many rat or mouse units per cubic centimeter. In his recent book Frank states that he has examined a number of commercial products and found many of them of much lower potency than was claimed. I have myself checked a few similar preparations and although it was possible to produce a reaction in most of the animals injected, it was very evident that they did not contain the required number of units. There are probably two explanations for this fact. First, biologic assay is an indefinite factor due to irregularity of response in laboratory animals, etc., and secondly, it is possible that many solutions deteriorate rapidly and so have lost much of their potency by the time they reach the consumer.

Two other objections arise. First, the preparations are very expensive, due to the complicated procedure required for their manufacture. Secondly, there is a real handicap in that most of the estrin available in this country must be administered hypodermically. This objection has been partly overcome since Pratt and Smeltzer have shown that it may be given with an intranasal spray, and since vaginal suppositories containing estrin seem to be a very effective method of administration. There is, however, a demand for preparations such as are in use in Germany and which may be given orally.

(b) *Dosage*.—The dosage is, of course, of vital importance and offers a very real problem

which is further complicated by the uncertainty regarding the absolute potency of the products in use. It would seem that very large doses must be given in order to assure a definite response in the pelvic organs. Frank and Goldberger have shown that in the premenstrual phase the blood may contain as much as one mouse unit of estrin per forty cubic centimeters of blood, while Siebke estimates that at this time the total amount in the blood is two hundred mouse units. The amounts found in the urine are also very remarkable, and Siebke in his extensive investigations found that as much as 1830 mouse units were excreted by the kidneys in the course of a twenty-eight day cycle. These figures would tend to show the importance of large dosage, and in his recent work with monkeys Allen gave as much as 80 units per day and a total of 1160 units per animal in order to produce the results he obtained. In the endometrium of a woman who has passed the menopause, I was unable to induce any histologic change, with 250 units of estrin given over a period of five days.

(c) *Time of Administration*.—Since the studies of Frank and Goldberger and Siebke have shown us that there is a variation in the amount of estrin found in the blood and that it reaches its maximum just before menstruation, it would seem that the administration of estrin should follow this plan. It should, therefore, be given in large amounts just before menstruation is expected, and Siebke in following this method has given it over a period of twelve days.

Considerable attention has been directed to Novak's suggestion that in conforming to the events of the normal cycle, estrin should be given and then followed by the administration of a corpus luteum hormone. This method has given marked success in certain animal experiments, but in the question of therapy it would seem that to follow the human cycle more closely one should combine the two suggested methods, that is, give estrin in small doses for a time and then in large doses along with the corpus luteum preparation. As the interaction of these two substances seems to be a very delicate quantitative problem it is likely that it will be some time before we know how to administer them accurately. It is possible that the solution will be found in a preparation of the corpus luteum which contains both substances in the proportions in which they normally occur in this structure.

(d) *Indications*.—The self-evident indication for the use of estrin is in women with ovarian hypofunction, and it must be regarded in the same light as insulin, namely, as a purely substitutive therapy. A careful diagnosis is an absolute prerequisite for success with this method of treatment since anatomical lesions of the pelvic organs and systemic diseases are frequent etiological factors and in such cases it is useless to resort to endocrine therapy without first attending to the primary condition.

Secondary ovarian deficiency may be said to manifest itself mainly in two ways: (a) nervous

symptoms—flushes, nervousness, headache, dizziness, vomiting—and (b) disturbances of menstruation—amenorrhea, scanty menses, lengthened intervals between menses, and possibly some cases of sterility.

(e) *Results*.—In analyzing the results that have been reported one cannot help but feel very disappointed and it must be acknowledged that this therapy has not come up to expectations. Many favorable results have, of course, been announced on the use of estrin in the treatment of nervous symptoms associated with ovarian deficiency and this is highly desirable because I feel that it is really the outstanding indication for the use of ovarian extracts. However, any estimation of clinical findings in such cases must be regarded with considerable skepticism and should be very carefully controlled, since there is always a pronounced psychic element entering the picture. We have also all seen good results in such patients by the use of simple sedatives such as bromids and luminal, and many of us regarded very favorably the dried ovarian extracts which have recently been condemned as biologically inert, so that some doubt arises as to whether the new active substances really represent a great advance. It is also likely that the problem may be faced from other standpoints. For instance, in the absence of ovarian function there results a hyperfunction of the anterior lobe which is possibly a factor in the production of symptoms, and instead of trying to supply the ovarian deficiency it may be preferable to diminish anterior hypophyseal activity by x-radiation.

In the treatment of menstrual abnormalities estrin has some value, but I feel that it must only be employed under certain definite indications. In women with persistently delayed menses who complain of sterility and in whom blood tests have shown a low amount of the hormone, it may conceivably be given just before the expected period. By this method it may be possible to stimulate the endometrium to more complete development and so afford proper nidation for the fertilized ovum. However, since it seems well established that these preparations cannot stimulate the ovaries themselves it is useless and illogical to give them in the hope of correcting the menstrual irregularity. This is also the case with patients complaining of functional amenorrhea. It is possible here to give large doses of estrin and induce a "menstruation" on discontinuing the administration, just as Allen did with castrated monkeys. There are also a number of reports that this has been accomplished in humans, and many authors have greeted the finding as a notable achievement. It is, however, an absolutely useless procedure since there is no accompanying ovulation and it cannot lead to conception. The absence of menstruation *per se* does not necessarily do harm, and I fail to see any logic in inducing an abnormal blood loss in these women. The cause of the trouble is more deep-seated and does not usually lie in the uterus itself, which is the organ affected by this type of therapy.

The use of corpus luteum extracts in the treatment of menorrhagia has long been advocated because of the clinical observation that the persistence of this structure leads to amenorrhea. In view of the newer studies which demonstrate the effect of progesterin on the endometrium, I fail to see just how it could control abnormal bleeding, but it will be necessary to wait until potent extracts of this substance are available for clinical purposes before a final verdict can be given. In the meanwhile there is some reason for employing estrin in these cases as it may stimulate proliferation of the endometrium and hence a more rapid tissue repair following desquamation. In the abnormal bleeding of puberty and the menopause, estrin is not only useless but is clearly contraindicated since most of these patients have a hyperplasia endometrii, a condition primarily due to an overproduction of estrin in the ovaries. Frank reports satisfactory immediate results in the treatment of kraurosis vulvae, but no permanent effect could be obtained.

B. *Anterior Hypophysis*.—In the sex hormones of the anterior lobe lie most of our hopes for the successful treatment of amenorrhea and delayed menses since we have here a powerful stimulant to the ovaries themselves. Instead of using a purely substitutive therapy we will then be initiating true ovarian and uterine function by direct stimulation. Time only will tell to what extent this will be accomplished. There is no doubt that it will be found to have many limitations. For instance, I have found by blood tests that some patients with prolonged amenorrhea already have an overproduction of anterior lobe hormone, so that it would be useless to hope for success in these women by administering further amounts of this substance.

There is as yet no potent biologic product of the sex hormones of the anterior lobe available for the profession at large, but there is no doubt that it will not be long before it can be procured. Some preparations have been made and clinical experimentation is in progress. The results obtained to date on the Stanford service are hopeful, but it is too soon to draw any conclusions.

There are numerous sources for the ovary-stimulating hormones now being tried. In Germany the blood serum of pregnant women has been used for direct injections, and a potent product (prolan) has been prepared from the urine of such patients. In this country a great deal of interest is focussed on the work of Professor J. B. Collip of McGill University, who has succeeded in obtaining an ovary-stimulating substance from the placenta (Emmenin). This preparation is active when administered by mouth, and preliminary experiments have been most encouraging. As has been suggested by this laboratory, it is also possible that the blood serum of castrated animals may offer a potent source for similar extracts. And finally, recent preliminary studies also point to the successful use of "luteinizing" extracts in the control of the uterine hemorrhage accompanying hyperplasia of the endometrium.

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